étudiées. La substitution en position 4 de la glutamine par la sérine diminue considérablement l'activité pressique de la vasotocine, ce qui montre l'importance de cette position dans les interactions entre les hormones neurohypophysaires et le récepteur vasopressique.

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29 May 1972.

[22] The authors are indebted to Dr. ALBERT JÖHL (Ciba-Geigy Laboratories) for a sample of synthetic Ser[4]-Arg[8]-oxytocin. They thank Mrs. CHRISTINE CAULLIER for ther skilled technical assistance.

Chromosome Replication in Cells of a Continuous Line Derived from *Aedes albopictus* (Skuse) Larvae

Over the past several years continuous cell lines derived from a number of mosquito species have been developed[1–9]. Although there is some data on the chromosome complement of some of these lines[1, 5, 7, 9, 10], it appears valuable to have the karyological characterization of some of these strains as complete as possible. In previous publications, we have reported data on the karyology of 3 cell lines derived from either *Aedes aegypti* or *Aedes albopictus*[10], and on the pattern of constitutive heterochromatin distribution in tissue culture cells from the latter species[11]. This paper will give information on the pattern of DNA synthesis by the chromosome complement of the *A. albopictus* cell line.

Studies were performed in passage 162 of a cell line (designated ATC-15) initiated from first instar larvae of the mosquito *A. albopictus* (Skuse) by SINGH[2] and grown on the medium described by him. Cultures in the log phase of growth were divided into 2 groups and respectively treated for 5 or 3 h with 1 μC/ml of 3HTdR (specific activity 6.9 C/m*M*). Both groups of cultures received 0.12 μg/ml of Colcemid 3 h before harvesting. Cells were removed by trypsinization, hypotonically treated and fixed in 3:1 ethanol:acetic acid. Chromosome spreads were obtained by air drying and stained with carbol fuchsin. Slides were mounted with AR10 Kodak stripping film and exposed for 10 days. Autoradiograms were processed and analyzed as described elsewhere[12].

The karyology of ATC-15 cells has been recently reported[10, 11], hence, it will be only briefly mentioned here. The chromosome complement is formed by 3 pairs of metacentric chromosomes. Following the usual nomenclature for mosquito chromosomes the longest and shortest pairs in the set are numbered 3 and 1, respectively. Approximately 48% of cells had chromosome aberrations ranging from chromatid gaps to open or rearranged chromosomal breakages. These aberrations were not random, but preferentially located in the proximal third of 1 arm in pair 1 and in the distal third of 1 arm in pairs 2 and 3. The incidence of polyploidy varied from 10 to 18%[10].

Autoradiograms obtained from the 5 and 3 h 3HTdR treatment showed 82 and 48% of labeled mitosis, respectively. Thus, a G2 period of approximately 3 h may be assumed for ATC-15 cells[13]. The amount of silver grains on labeled metaphases was variable and allowed one to assemble the cells in a series of continuous decreasing radioactivity. Metaphases at the beginning of the series showed labeling all over the chromosome complement (Figure 1). In further stages the deposition of silver grains on the chromosomes decreased and the absence of labeling in the pericentromeric areas of pairs 2 and 3, and occasionally, in the middle third of 1 arm in pair 1 was noticed (Figures 2 and 3). Afterwards, unlabeled areas increased in extent and in metaphases at the end of the series labeling was restricted to: a) the upper half of one arm and the distal third of the other in pair 3; b) the distal half of both arms in pair 2; c) the distal third of one arm and the proximal third of the other in pair 1; d) centromere of the 3 pairs (Figures 4 and 5). Patterns c) and d) were less constant and conspicuous than patterns a) and b).

The heaviest labeled metaphases arose mainly from cultures treated with 3HTdR for 5 h. On the other hand most of the metaphases with less than half of the complement labeled stemmed from the 3 h treatment. In cells continuously treated with 3HTdR unlabeled chromosome areas represent the genome regions which have finished replication before the isotope was added. Thus, by treating different cell populations for variable time lapses it is possible to obtain a sequence of labeling patterns representing various stages of the S phase. In the foregoing series, complete labeled metaphases probably arose from intermediate stages of the S period in which most or all replicating units in the genome are engaged in DNA synthesis. On the other hand, radioactive regions from partly labeled metaphases illustrate the areas involved in replication during the late and final stages of the S period (late replicating regions).

It is presently held that late replication is one of the most conspicuous properties of heterochromatin[14]. Ac-

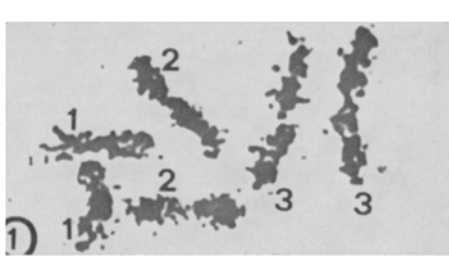

Fig. 1. Autoradiogram showing labeling all over the chromosome complement. Chromosomes are numbered. All photographs × 1,500.

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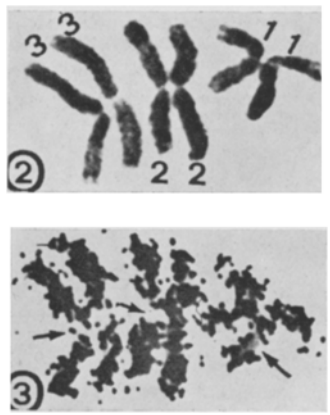

Fig. 2 and 3. Metaphase and corresponding autoradiogram showing lack of labeling in the pericentromeric regions of pairs 2 and 3 (arrows) and in the middle third of one arm in pair 3.

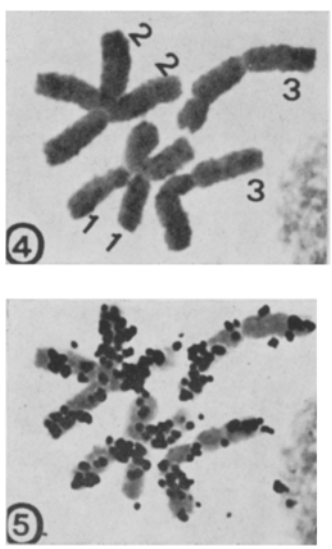

Fig. 4 and 5. Metaphase and corresponding autoradiogram showing the location of late replicating regions.

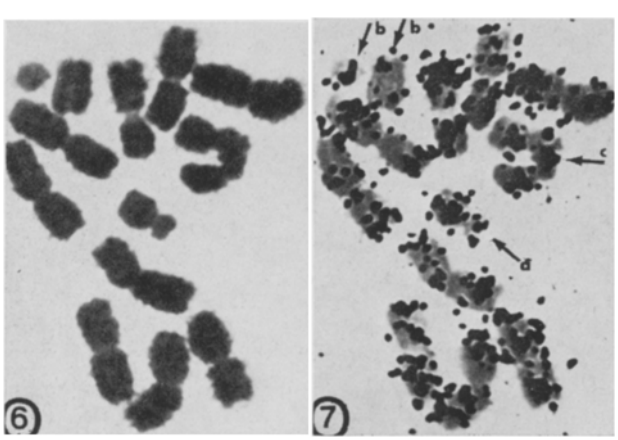

Fig. 6 and 7. Polyploid metaphase and corresponding autoradiogram showing late replication in the areas of chromosome breakage and rearrangement (arrows): a) deleted chromosome, b) 2 acentric fragments, c) dicentric chromosome with late replication in the area of chromo- some fussion.

cordingly, it seemed of interest to compare the location of late replicating areas with that of the constitutive (c) heterochromatin. In ATC-15 cells, using the DNA denaturation-renaturation (DNA d-r) method, we have shown the presence of c-heterochromatin in the centromeric region of the 3 chromosomal pairs and in the proximal third of one arm in pair 1[11]. Since all these regions are late replicating, c-heterochromatin in ATC-15 cells may be considered to exhibit asynchronous DNA synthesis. Conversely, distal areas of both arms in pairs 2 and 3 clearly show the existence of late replicating areas not associated with c-heterochromatin. It has been reported lately that c-heterochromatin comprises highly repeated DNA sequences[15] which are responsible for the differential stain obtained with the DNA d-r method[16]. On the other hand, facultative (f) heterochromatin is apparently formed by non-repeated DNA sequences which do not react with the DNA d-r method[17,18]. It may then be surmised that the distal regions of both arms in pairs 2 and 3 may represent the location of the f-heterochromatin in the genome.

In metaphases with aberrations the areas of chromosome breakage were always labeled, even in those cells exhibiting small amounts of radioactivity (Figures 6 and 7). This finding may be explained by assuming that: a) breakages were produced by the radiations of the isotope incorporated into the chromosomes, or b) breakages occurred in late replicating regions independent of the radiations. The finding of non-radioactive metaphases with breakages, and the spontaneous appearance of aberrations in ATC-15 cultures not tagged with 3HTdR[10] allow one to favor the second hypothesis; thus suggesting a strict correlation between heterochromatin and chromosomal aberrations in ATC-15 cells[19].

Resumen. El análisis del cuadro de duplicación cromosómica en una linea celular derivada del mosquito *Aedes albopictus* (Skuse) reveló un período G2 de 3 horas y la presencia de varias areas de duplicación tardía. La mayor parte de estas aread correspondieron a la heterocromatina constitutiva. Sin embargo, la porción distal de ambos brazos en los pares 2 y 3 mostraron duplicación tardía no asociada con la heterocromatina constitutiva. Se sugiere que estas regiones corresponden al a heterocromatina facultative.

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